Report of the Ninth Meeting of the Scientific Review and Technical Advisory Committee of the Cholera Research Laboratory

Dacca, Bangladesh

February 25 - March 1, 1975

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Report of the Ninth Meeting of the Scientific Review and Technical Advisory Committee of the Cholera Research Laboratory Dacca, Bangladesh February 25 - March 1, 1975

Agenda and Participants

The Technical Committee met at the CRL in Dacca on Tuesday and Wednesday, February 25 and 26, in Joint Session with the ad hoc site visitors to the Johns Hopkins University Center for Medical Research (CMR) to hear presentations of studies carried out by CMR scientists who are guest investigators at the CRL.

On Thursday, February 27 and Saturday, March 1, the Technical Committee met at the CRL in Dacca to hear reports of scientific activities presented by the members of the CRL staff.

On Friday, February 28, the Committee visited the Vaccine Trial Study Area at Matlab Bazar, where the results of some of the field studies were presented.

On Monday, March 3, the Committee met in Dacca in Joint Session with the Directing Council of the Cholera Research Laboratory.

In addition to the members of the CRL staff and the Johns Hopkins CMR investigators, the following persons were in attendance at these meetings:

Members of the Technical Committee

Dr. John P. Craig
Professor of Microbiology and Immunology
Downstate Medical Center
State University of New York, Brooklyn, New York, U.S.A.
Chairman of the Technical Committee
Government of the United States of America

Dr. M.A. Latif
Deputy Director
Biological Production Laboratory
Institute of Public Health, Dacca, Bangaldesh
Government of Bangladesh

Professor Derrick Rowley
Professor of Microbiology
University of Adelaide
Adelaide, Australia
Government of Australia

Professor R.E.O. Williams
Director, Public Health Laboratory Service, London, England
Government of the United Kingdom

Expert Advisors to the Technical Committee

Dr. Philip S. Brachman
Director, Epidemiology Program
Center for Disease Control
U.S. Department of Health, Education and Welfare
Atlanta, Georgia, U.S.A.

Dr. Edward W. Hook, Jr.
Professor and Chairman
Depratment of Medicine
University of Virginia School of Medicine
Charlottesville, Virginia, U.S.A.

Dr. Nevin Scrimshaw
Head, Department of Nutrition and Food Science
Massachusetts Institute of Technology
Cambridge, Massachusetts, U.S.A.

Observers

Dr. K.A. Monsur
Director, Institute of Public Health
Dacca, Bangladesh

Dr. Earl S. Beck
Head, Geographic Medicine Branch
National Institute of Allergy and Infectious Diseases
Bethesda, Maryland, U.S.A.

Dr. Carl Miller
Cholera Program Officer
Geographic Medicine Branch
National Institute of Allergy and Infectious Diseases
Bethesda, Maryland, U.S.A.

Dr. Bradley Sack
Co-Director, Johns Hopkins University Center for Medical Research
Associate Professor of Medicine
The Johns Hopkins University School of Medicine
Baltimore, Maryland, U.S.A.

In addition the following ad hoc site visitors to the Johns Hopkins University Center for Medical Research attended those portions of the CRL program review which involved CMR scientists:

Dr. Henry Riecken
Professor of Behavioral Sciences
School of Medicine
University of Pennsylvania
Philadelphia, Pennsylvania, U.S.A.

Dr. John Scanlon
Professor of Medical Zoology
School of Public Health
University of Texas
Houston, Texas, U.S.A.

Dr. Robert Shope
Director
Yale Arbovirus Research Unit
Yale University School of Medicine
New Haven, Connecticut, U.S.A.

Dr. Paul Weinstein
Professor and Chairman
Department of Biology
University of Notre Dame
Notre Dame, Indiana, U.S.A.

Dr. Kenneth Phifer
Executive Secretary, ICMR Program
Geographic Medicine Branch
National Institute of Allergy and Infectious Diseases
Bethesda, Maryland, U.S.A.

INTRODUCTION

The Cholera Research Laboratory was officially re-established under bilateral agreement between the Governments of Bangladesh and the United States of America in May, 1974. This meeting of the Technical Committee represents the first scientific review of the CRL since the independence of Bangladesh, although the CRL continued to operate without interruption as a clinical facility since the last meeting in 1970.

The committee wishes to recognize and applaud the dedication and perseverance of both the Bangalee and expatriate staff members whose efforts and loyalty through the recent difficult years made possible the survival of the CRL as a vigorous and creative institution. Special gratitude must be extended to Drs. Kendrick Hare, the most recent Director of the CRL, and to Dr. Ruth Hare and Dr. Mujibir Rahman and Mr. Mark Tucker all of whom risked their lives in their successful effort to save the CRL during the recent war and the turmoil which occurred in its aftermath.

The committee also wishes to recognize and applaud all those who supported and worked in the CRL while its destiny was uncertain before its official re-establishment last year. The committee wishes to compliment Dr. George Curlin for his excellent performance as Acting Director of the CRL in 1973-74 before the re-establishment. His efforts provided a smooth transition from old to new under difficult circumstances, and laid the foundation for a re-vitalized CRL under the terms of the new agreement. He was able to inaugurate a number of new scientific programs which have been very successful. The committee also wishes to commend Dr. Willard Verwey who assumed his duties as Director of the CRL in June, 1974, for

his success in launching the CRL under its new administrative framework, and for inaugurating and sustaining a scientific program of generally high quality in the face of great difficulty and with a very markedly reduced expatriate staff. We should also note that the resources and facilities of the CRL were severely strained during the entire latter half of 1974 by an unusually early and unusually severe epidemic of cholera in both Dacca and Matlab Bazar. During this time the CRL hospital in Dacca admitted and treated more patients than in any year of its existence.

In view of these many problems with which the Director and his staff have had to cope, the committee feels that the scientific output of the CRL during the first seven months of existence under its new administrative framework has been of generally high quality, and has, with few exceptions, been aimed at appropriate goals.

During the past two years a special relationship has developed between the CRL and the Johns Hopkins University Center for Medical Research (JHCMR) which now resides in Dacca under agreement between Johns Hopkins University and the Government of Bangladesh. The staff of the JHCMR is quartered in the Bangladesh Institute of Public Health adjacent to the CRL and that portion of the JHCMR which is conducting studies of diarrheal disease and subjects related to CRL research programs are actually accommodated within the CRL organization as guest investigators. Thus three U.S. investigators who are members of the JHCMR staff are considered to be CRL guests and their research is considered in this report as a part of total CRL output.

Scientific review of the JHCMR program was conducted concurrently by an ad hoc group of project site visitors from the NIH, U.S.A.

The Technical Committee is grateful for the assistance and continuing interest of our expert advisors. The fine cooperation and assistance of the Director and his staff have made our review pleasant as well as informative. This report attempts to review briefly the different investigative areas in which the CRL is engaged, and does not necessarily deal with each presentation made during the meeting.

Review of Scientific Activities

I. Physiology Division

A. Clinical Trial of Doxycycline in Cholera

The efficacy of doxycycline, a long-acting tetracycline, was compared with a placebo or tetracycline hydrochloride in 51 patients with cholera. The patients receiving doxycycline purged a mean volume of 5.1 liters of stool and received an average of 5.7 liters of intravenous fluids compared to 10 liters of stool and 9.7 liters of intravenous fluids required by the patients on placebo. In comparison, the patients receiving tetracycline purged 4.8 liters of stool and received 5.5 liters of intravenous fluids. The mean duration of diarrhea was 3.5, 8 hr periods in the doxycycline group; 4.1 in the tetracycline group; and 8, 8 hr periods in the placebo group.

The difference between the doxycycline and placebo and between tetracycline and placebo were both statistically significant. Bacteriologic examination showed that the tetracycline group became negative for vibrios within two days as compared to three days in the doxycycline group and a much longer period in the placebo group.

The results show that doxycycline administered once daily was as effective as tetracycline given every six hours in the treatment of cholera.

These results are not surprising. Tetracycline, irrespective of type, is effective in cholera. The additional cost does not justify the use of doxycycline as the antibiotic of choice in cholera as compared to tetracycline.

B. Comparative Trial of Dacca Solution, and a low Sodium-Containing Solution in Intravenous Therapy of Noncholera Diarrhea

Intravenous therapy with Dacca solution containing 133 mEq of sodium as well as potassium, bicarbonate, and chloride was compared with a low sodium solution, called the "diarrhea" solution, which contained 116 mEq of sodium. The results showed that there was no appreciable difference in the rate of correction of dehydration or serum electrolytes between the two groups.

C. Oral Sucrose Studies

Studies were reported on the substitution of sucrose for glucose in oral fluid replacement therapy for cholera. Sucrose content was 40 g/liter, yielding 20g glucose and 20g fructose which purportedly does not enhance sodium absorption. In a small study of about 15-18 patients, 3 individuals showed an increase in stool volume and i.v. fluids had to be used to achieve adequate rehydration. The majority of patients, however, responded as well to sucrose as to glucose. Based on this finding and supported by the fact that disaccharidase activity may be impaired in acute diarrhea, no further studies have been planned.

The committee, however, feels that oral sucrose merits further study because of its potential practical worldwide usefulness if it should prove to be an effective substitute for glucose. Sucrose is universally available.

We feel that the number of patients studied thus far is too small to warrant rejection of such a potentially useful procedure. It seems to us desirable to carry out a properly controlled study on at least 200 cases of acute diarrhea using glucose, sucrose and perhaps a third oral group with electrolytes only (which has never been done). Such a study would be safe, inexpensive, and could offer data of very high applicability.

D. Inhibition of Fluid Losses by Intraluminal Acid

Studies have been continued on the effect of zirconium oxychloride and luminal hydrochloric acid on enterotoxin-induced fluid and electrolyte losses in acute small bowel loops in dogs. It has been demonstrated that the inhibition of fluid loss was caused by low pH rather than zirconium and could be reproduced by HCl as long as the same low pH could be achieved.

Histological studies on dog small bowel mucosa subjected to this treatment have shown that HCl causes complete loss of villous tips with suprisingly rapid pinching off of villi followed by healing and re-epithelialization. There is no detectable blood loss, although there is marked increase in protein in the small bowel fluid. It is assumed that reduction in fluid loss is due to removal of toxin-activated cells which are hypersecreting.

A protocol has been submitted for testing the therapeutic value of this procedure in volunteers in Bangladesh. Treatment will begin with 25 mM HCl, about 1/2 liter per hour and work up to 100 mM if there are no adverse effects. The investigator points out that earlier gastroenterologic studies in volunteers have involved intraluminal installation of similar concentrations of HCl in duodenum and in mid and lower small bowel without untoward consequences. In order to be carried out in Dacca this

protocol will have to be approved by the Clinical Research Review Committee of the Cholera Research Laboratory and the Bangladesh Medical Research Council.

It was previously recommended by the JHCMR visiting committee that no human studies be carried out until this procedure has been tested in intact dogs challenged with living vibrios. Live challenge studies have still not been done, and our committee agrees that these are required. There is some feeling that this procedure would contaminate the effluents from the animal house with Vibrio cholerae because isolation and sterilization procedures for effluents are inadequate. Nevertheless we feel that if this procedure is really worth trying in man, it demands more complete study in the intact infected animal which would simulate cholera in man more closely than do acute ligated bowel loops challenged with toxin alone.

However, we doubt that HCl therapy could be developed into a practical procedure for treatment of large numbers of cases in an epidemic situation or in untrained hands. Cholera therapy is now very satisfactory when materials and personnel are available. What is needed is a cheap and safe method of rehydration and/or reducing fluid output which can be administered on a mass scale with little supervision. Oral therapy has contributed to this end in a major way and efforts should be continued to improve oral therapy materials and techniques. We feel that HCl is unlikely to have comparable usefulness even if it proves to be experimentally successful.

E. Leukemoid Reaction and Hemolytic Anemia in Shigellosis

An exceptionally large number of cases of shigellosis have been admitted to the CRL during the past few years, in many seasons outnumbering admissions for cholera.

During the epidemic of Shigella dysenteriae Type 1 (Shiga Bacillus) infection in Dacca over the past two years, leukemoid reactions with white blood cell counts greater than 50,000 cells per mm^3 have been observed in about 15 percent of the patients. One-third to one-half of the patients with leukemoid reactons developed acute hemolytic anemia associated with erythrocyte fragmentation visible on blood smears. Most patients develop thrombocytopenia. Abnormalities of renal function occurred and several patients had frank renal failure. Toxic megacolon and nephrotic syndrome have been described.

Research aims were to define the pathogenesis of this syndrome and explain its high incidence. Progress during the past year has not been impressive.

New observations have been as follows: (1) red blood cell fragmentation, with occasional burr cells and microspherocytes, is common; (2) fibrin split products are present; (3) hypothrombinemia and hypofibrinogenemia are associated with the hemolysis; and (4) the prothrombin and prothrombin time (PTT) are not prolonged. Several of these findings are consistent with intravascular coagulation, but the lack of consumption of clotting factors (normal prothrombin and PTT) is against this possibility. Additional assistance from experts in hematology and blood coagulation should be obtained.

The investigators have failed adequately to characterize the clinical syndrome utilizing simple, readily available techniques, despite the fact that the importance of obtaining these basic clinical data was pointed out at the JHCMR site visit one year ago. For example, the frequency of bacteremia and the time of onset of hemolysis in relation to the onset of diarrhea should

be determined. Samples of blood were obtained from 43 shigellosis patients for T and B cell studies, yet very few blood cultures have been done. It is still not clear whether hemolysis occurs during the acute phase of the disease or is a late complication.

Hemolysis could be the result of the acute infectious process in patients with hematologic abnormalities, such as abnormal hemoglobins or glucose 6PD deficiencies. These possibilities could be tested quite easily. Finally, microangiopathic hemolytic anemia has been described in association with a number of disorders in which red blood cells are mechanically fragmented by contact with abnormal small blood vessels (e.g., vasculitis). Since the exotoxin of Shiga's bacillus acts on small blood vessels, it would seem important to consider the possible influence of a toxin which might attach to erythrocytes or endothelium. Toxin production by epidemic strains could and should be studied.

F. T and B Cell Responses in Shigella Infection

This study was designed to determine the response of T and B lymphocytes in patients with shigellosis, especially those with Shigella dysenteriae Type 1 (Shiga's bacillus) infections with leukemoid responses. Forty-three patients have been tested to date and the percentage of T and B lymphocytes estimated by standard techniques.

The results indicate that there is a depression of the percentage of T cell rosette-forming lymphocytes. Depression of T cells below 40 percent was demonstrated in 32 of 43 samples; the percentage of T cells should exceed 50 percent under normal conditions. There was no significant change demonstrated in the proportion of B cells.

A large population of cells could not be counted as either T or B cells. The usual proportion of such "nil" cells is around 3 percent but in patients with shigellosis with leukemoid reactions the percentage of "nil" cells amounted to 10 to 60 percent of the total lymphocytes. This requires further definition; it is possible that these cells represent another type of lymphocyte, immature lymphocytes, or some heretofore undescribed cell.

This study contributed to information about shigellosis. However, the study has not been "controlled" with comparable studies in patients with shigellosis caused by other Shigella species,with shigellosis without leukemoid reactions, or with diarrhea due to other causes.

The committee felt that the present data are difficult to interpret in the absence of information related to nutritional status. The percentage of lymphocytes is known to be depressed in malnutrition. Nutritional status of the patients must be considered in the interpretation of these results.

G. Nutritional Status and Severity of Diarrhea in Cholera

Several previous studies have indicated an association between malnutrition and diarrheal diseases, including cholera. The goal of the present study was to define the influence of nutritional status on severity of diarrhea in cholera. Ninety-seven children and adults with bacteriologically-confirmed cholera were studied during the fall 1974 epidemic.

The influence of tetracycline on the course of illness was re-evaluated. The volume of stool was measured for each eight-hour period, and the duration of diarrhea was defined as the period of time up to the first eight-hour period during which there was no stooling. Nutritional status was evaluated

just prior to discharge utilizing weight-for-height and the Matlab standards. Nutritional status was quite variable; there was some marasmus but no kwashiorkor.

Tetracycline was again shown to be effective. The duration of diarrhea averaged 72 hours in the group receiving no antibiotic and 34 hours in the tetracycline-treated group.

The new observation was the correlation between malnutrition and duration of stooling. The longer duration of diarrhea in poorly nourished patients was observed in the group treated with tetracycline as well as in the group without tetracycline. Stool volume per unit of time was not influenced by nutritional status in either group.

The mechanism of this phenomenon has not been defined and several explanations are possible. However, the most popular is based on the facts that (1) once cholera enterotoxin is fixed to a cell it continues to exert its pharmacologic effect throughout the life of the cell, and (2) the life of mucosal cells is prolonged in patients with malnutrition. Thus, it is postulated that the effects of nutrition in prolonging cholera diarrhea may be related to slower mucosal epithelial cell proliferation in malnourished patients. The effect of enterotoxin is prolonged in malnourished patients because the functional life span of the cell is prolonged.

The committee found these studies of interest and of high quality. There was some concern that the investigators decided to re-evaluate the effect of tetracycline therapy in cholera. It should have been possible to show the influence of nutrition in tetracycline-treated patients, and the untreated control group was not necessary. The method of calculating

duration of stooling was criticized because it is likely this method led to error in estimating the duration of diarrhea. However, since the same method was applied to all groups, the error was probably evenly distributed.

H. Influence of Nutrition and Immunological Status on Morbidity from Diarrhea

The increased incidence of infection among malnourished individuals is a well-established concept but the mechanisms by which malnutrition influences infection are inadequately defined. The goal of this proposal is to determine whether impaired cell-mediated immunity (CMI) can be related to infection in malnourished children.

The study group consists of approximately 200 children in the Matlab study area of the CRL. Basic data regarding height, weight and age have been obtained and the group is to be followed carefully for occurrence of diarrhea utilizing the well-established surveillance system operating in Matlab. The plan is to carry out skin tests with common antigens including tuberculin, streptokinase-streptodornase and candidin. In addition, skin sensitization studies with dinitrochlorobenzene will be carried out, and finger prick bloods will be collected for serum proteins and antibody titers.

The preliminary results offer some evidence for a correlation between anergy and diarrhea attack rate.

The measurements selected are the most practical ones and are entirely appropriate. There needs to be more analysis of the data, particularly on the effect of different classifications of nutritional status on the significance of the differences observed. When more data have been accumulated,

a comparison should be made between the percentile system and the Gomez system of defining degrees of malnutrition from weight-for-age data. Since finger prick blood is being obtained, it would be worthwhile to determine red cell protoporphyrin as a measure of iron status, and serum vitamin A levels.

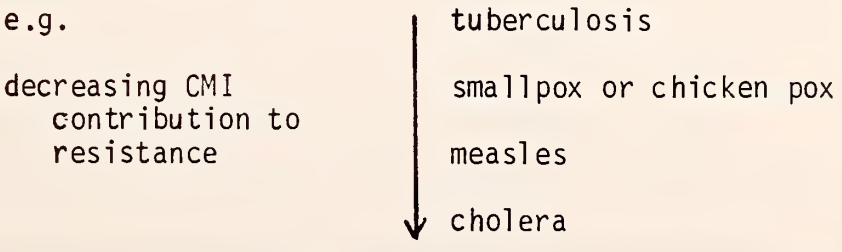
These are well-conceived and apparently well-executed studies. They are highly relevant and can be carried out only because they are founded on the years of development of the Matlab surveillance system. For this reason these needed studies are unlikely to be done as well anywhere else in the world, and should be encouraged and supported.

Some of the more obvious questions are:

1. Is any one nutritional factor more important than others in determining susceptibility, such as protein nitrogen, iron or vitamins?

2. Can the increased incidence of diarrhea diseases be correlated with any non-immunologic factors such as gastric acidity, frequency of food intake, peristalsis, structure of jejunal mucosa?

3. Does depression of CMI have its major impact on those diseases in which CMI plays a major role in resistance? Are the effects of malnutrition most marked in those diseases:



If data from the current field studies confirm the initial indication of a relationship between malnutrition, impaired CMI and morbidity from diarrheal and other infectious diseases, the demonstration of a causal

relationship among the three will still be needed. This can be accomplished by an intervention study in the same population in which at least 100 children would receive a daily supplement of nutritious gruel, while a matched group would receive no supplement. This could probably be arranged through the same village dais who now conduct surveillance. The minimum time for meaningful data would probably be six months.

Further consideration should also be given to the best method of obtaining morbidity data. Visits every three days may not be necessary. Even if seven days are less reliable, it might be better to use longer intervals on a larger population so long as the data were comparable throughout the study. Consideration should also be given to a pictorial scheme which would allow the dais to collect morbidity data from their daily visits.

I. Cell-Mediated Immune Response in Acute Infection

Depression of cell-mediated immunity (CMI) has been noted in a number of viral and bacterial infections. In addition, anergy has been reported to be associated with leukocytosis of diverse causes.

The purpose of the present study is to determine the influence of a number of acute infections, but especially cholera, on delayed hypersensitivity and CMI. Patients to be tested are those admitted to the CRL hospital in Dacca. Smallpox cases will also be studied at the nearby Infectious Disease Hospital. The patients will be tested on several occasions during illness and convalescence with standard intradermal skin tests using ubiquitous antigens (tuberculin, candidin, streptokinase-streptodornase). The capacity of DNCB to sensitize these patients will also

be tested.

Thirteen children and 16 adults with cholera have been tested to date. A control group consisted of 17 healthy adult Bengalees. The response rate in the cholera patients is less than half that of normal controls.

Because of the demonstrated immunosuppressive effect of cholera toxin, the goals of this study seem appropriate. However, there is a question whether the investigators can adequately control the experiments. In the first place, the majority of patients with cholera are malnourished; malnutrition in itself could account for depressed CMI. The investigators feel that the role of malnutrition can be evaluated with an "appropriate" group of controls but it is uncertain whether "controls" selected on the basis of comparable height and weight but without diarrhea will represent equivalent nutritional status.

In addition,the investigators propose to have each patient serve as his own control. That is, patients will be retested during convalescence and if anergy to ubiquitous antigens disappears upon recovery it will be assumed that anergy was related to cholera. The problem with this "control" is that during therapy for cholera, nutritional status will also be improving and it will be difficult to determine whether cholera or malnutrition depressed CMI. Finally, a "nonspecific" depression of skin reactivity occurs in malnutrition and must be separated from any specific immuno-suppressive effect.

The committee pointedout that because of the difficulties in providing for adequate controls in this study, it is unlikely that it will be possible to determine conclusively whether cholera depresses CMI.

J. Teknaf Shigella Project

A protocol was presented for conducting a study of the treatment and epidemiology of shigellosis in Teknaf, a town of about 75,000 in southern Chittagong. This project will be funded by UNICEF. The study was prompted by a severe outbreak of Shiga dysentery there in 1973. The proposal aims to determine the incidence and types of shigellosis, and the factors influencing transmission, susceptibility and severity and to develop methods of intervention in transmission of shigellosis in a rural population.

Treatment facilities and laboratory were established in 1974, and it is proposed to develop a simple and effective method of therapy which may be applicable to the rest of the country.

This project had just been started at the time of our meeting, and it was therefore too early to assess results. However, the protocol that we have seen appeared to be too vague and to be over ambitious in that it proposed work of much greater scope than can be accomplished by the resources available. The great distance between Dacca and Teknaf puts a strain on the commuting staff. Some members of the committee wondered whether shigellosis studies might not have been more usefully integrated with the work on cholera and "culture-negative" diarrhea in Matlab. In other words, would it not have been better identified as a diarrheal disease study in which shigellosis would be but one of the recognizable causes? This is particularly important if it is to include children under one year of age, in whom E. coli are likely to be significant.

We doubt that it would have been wise to invest CRL funds in this

project. However, since it will be paid for by UNICEF funds and since shigellosis is an important national public health problem in Bangladesh, there is a justification for giving this project an opportunity to see what it can achieve during the coming year.

II. Epidemiology Division

A. Bacteriology

1. Cholera

An extensive study has been made of four culture media recommended for the isolation of Vibrio cholerae from feces or rectal swabs. The best proved to be Monsur's TTGA medium (taurocholate tellurite gelatin agar) but the other three all gave around 97% of the positives found on TTGA. For field conditions the TCBS medium might be preferred.

Bacteriologic examination is carried out on rectal swabs from all patients admitted to the CRL hospital. This has shown a remarkable change in the prevalent biotype of vibrio over the last 5 years, from a dominance prior to 1970 of "classical" Inaba, through "classical" Ogawa to El Tor Ogawa, with a substantial proportion of El Tor Inaba by 1973. Bangladesh thus continued to have a high prevalence of the classical biotype long after the start of the current pandemic of the El Tor biotype.

The detailed investigations of epidemiology and of environmental contamination with V. cholerae envisaged for the future will demand the use of more detailed type identification of the strains isolated and we would suggest that some of the effort at present devoted to the routine identification of strains in the hospital might be diverted to research on typing methods.

2. Shigella

Since 1971 there has been about a 10-fold increase in the number of patients coming to the CRL hospital with diarrhea found to be associated with Shigella infection; during 1973 over half the cases were due to Sh. dysenteriae Type 1 (Sh. shigae), most of the rest being due to Sh. flexneri.

3956 strains of Shigella have been tested for antibiotic resistance. Most strains of Sh. dysenteriae Type 1 were found to be resistant to sulfonamide, tetracycline and streptomycin; about 30% of the strains were also resistant to chloramphenicol. Antibiotic resistance was less frequent in Sh. flexneri strains. In both species an increase in the proportion of resistant strains was observed between 1971 and 1974.

It was felt that additional information regarding antibiotic usage patterns in the community would be helpful in evaluating the results of the antimicrobial sensitivity studies on shigella strains. One staff member suggested that approximately 80 percent of the patients presenting to the Cholera Research Laboratory with diarrhea give a history of previous antimicrobial therapy but others disagreed. A combination of chloramphenicol and streptomycin apparently is very commonly used by the citizens of Dacca. The committee felt that some better definition of this problem was in order.

The laboratory investigated a sharp epidemic of dysentery on St. Martin's Island, due to Sh.dysenteriae Type 1 and provided good evidence for its introduction by a single visitor from the mainland of Teknaf nearby. They have now set up, with the help of funds from UNICEF, on-going surveillance

in Teknaf where there is a continuing high incidence of dysenteriae and flexneri dysentery. This project has among its objectives, testing the relation of dysentery to nutritional status, cell-mediated immunity and water-supply. (see above)

During the shigella outbreak in Dacca it was observed that a proportion of the patients exhibited a "leukemoid" reaction, with a very high level of circulating leucocytes, often complicated by intravascular haemolysis and leading to death in several cases. Studies relating to this phenomenon are discussed above.

Shigellosis is clearly a very important constituent of the diarrheal disease problem in Bangladesh. Not only do the numbers of cases make up a substantial, and apparently increasing proportion of the patients seen at the hospital, but also the disease itself can be more severe than cholera in many cases. Work on the treatment, control and prevention of shigellosis therefore deserves strong support. This should include a more extensive surveillance system aimed at defining sources of infection, mode of spread, and host factors as well as laboratory studies relating to mechanisms of pathogenicity.

The only other major epidemic of Sh. dysenteriae Type 1 of recent years occured in Central America in 1970 and a search should be made for evidence on the relation of that epidemic to the present Bangladesh epidemic.

In the laboratory, there appears to be an excellent opportunity for investigating toxin production by the Sh. dysenteriae Type 1 strains. Blood cultures should also be examined from patients with and without the leukemoid reaction.

3. Toxinogenic Escherichia coli

In all studies of the etiology of diarrhea, a substantial proportion of patients fail to yield any of the recognized pathogenic bacteria: Vibrio cholerae, Shigella or Salmonella. Work elsewhere has shown that some strains of Escherichia coli can produce enterotoxin, and may be a cause of diarrhea. A series of patients, admitted to the Matlab hospital, from whom V. cholerae could not be isolated during the first few days of illness were examined for the presence of toxinogenic E. coli. Full clinical data have been collected on 50 patients and preliminary results indicate that about one third of these yielded toxinogenic E. coli.

There are at present two in vitro methods of demonstrating the heat labile E. coli enterotoxin, and one of these, the Chinese hamster ovary cell culture, has been established and validated at CRL, and will be applied to the Matlab strains.

Exploration of the possible role of E. coli in "culture-negative" diarrhea is certainly important, although fraught with difficulties of interpretation. There is always the possibility that the absence of pathogens may be due to antibiotic treatment outside hospital; perhaps the determination of antibiotic levels in urine would be useful. There is as yet far too little background information on the relevance of the in vitro demonstration of toxin to disease production in man. Moreover, as already seen in the Matlab studies, patients may be infected with potentially diarrhea-inducing parasites.

One interesting finding was the recognition of two strains of E. coli from man which appear to make only heat-stable enterotoxin. Toxic effect is short-lived (as compared to heat-labile toxin) and can be rinsed out when

tested in acute intestinal loops in dogs. This is the first recognition in Bangladesh of an E. coli strain which produces only heat-stable toxin.

The committee urges continued study of enterotoxinogenic E. coli at the CRL, and recommends broadening the toxin studies to include the suckling mouse assay, which now seems to be the method of choice for the detection of heat-stable enterotoxin.

B. Epidemiology

The study of urban cholera in Dacca in 1974 was well planned and carried out. It generated interesting data which has been well analyzed and presented. The route of transmission during this epidemic is not clear. It is of special interest that the epidemiologic data do not incriminate water supplies but do demonstrate that eating away from home was associated with greater risk of acquiring cholera. In spite of the lack of confirming epidemiologic data, however, water must still remain as the most likely method by which cholera vibrios were spread throughout the city. The suggestion of the role of food in transmission certainly deserves further study, however. Epidemiologic differences from reports of previous urban epidemics may be related to the effect of flooding or other unknown factors.

The number of cases of cholera admitted to the CRL hospital in Dacca affords a very sensitive indicator of the onset of an epidemic and could be used to trigger the initiation of epidemiologic studies. A protocol could be designed which might answer the questions not answered in this last study and which could be implemented at the appropriate time.

One area that may be worth investigating would be the environment, specifically the water supplies and possibly sewage systems. Some systematic plan to culture these areas may provide useful data for answering additional questions concerning urban cholera.

During the 1974 epidemic, data were collected from all parts of Dacca city. We would recommend that in future studies, a smaller area be selected for more intensive epidemiologic work. It should be an area large enough to provide an adequate population base, but small enough to allow an intensive study to be conducted. This study should include data concerning water and food source and consumption, family movement, secondary attack rates and nutritional status.

The data base provided by the CRL, namely the records on 18,610 bacteriologically confirmed cholera patients between 1964 and 1974 represents unique and invaluable data. An analysis of risk factors associated with death may help identify ways of preventing death. However, any retrospective study must be carefully planned. Of special importance is the inclusion of a control group, appropriately matched for certain factors, so that the resulting data have epidemiologic validity.

Much is known about the epidemiology of cholera; however, many questions remain unanswered. The vast opportunies to continue these studies must be utilized to the fullest possible extent. This includes Dacca as well at Matlab. The study done last year on defining cholera "hot spots" in the Matlab area showed great imagination and persistence of effort. Reviewing cholera cases for the past eleven years and pinpointing their location within Matlab has defined some areas with a relatively high incidence of cholera. To date the data from several of these "hot spots" have been analyzed and their association with canals is apparent. Analysis of the data from the other areas should be completed as soon as possible. The use of "water watchers" has provided further data that raise questions

concerning the exact role of water in the transmission of cholera.

These studies should be continued next cholera season. A protocol should be developed which should include careful collection of a variety of water samples for laboratory analysis for the presence of cholera vibrios. The use of "water watchers" raises certain problems; nevertheless, it may be important to repeat this aspect of the study and to couple it with a determination of the source of water within the home. This might be done by determining electrolyte content by conduction studies using simple portable testing equipment.

Studies of risk factors in cholera-infected baris were conducted during the last epidemic by visiting each bari from which cholera patients came for 10 consecutive days following the admission of the first case. Epidemiologic data as well as cultures were obtained from bari members. Interpretation is not yet possible because the data are still being analyzed. However these detailed studies should be extremely important in furthering our understanding of the epidemiology of cholera. Depending on the final analysis of these data additional studies may be conducted during the next cholera season. If risk factors can be identified, appropriate intervention studies can follow.

C. Demography

The data base provided by the ongoing collection of demographic data from Matlab provides a source of vital statistics from which many important analyses have been and can be made. Future studies of cholera and other enteric diseases, nutrition, immunity and population dynamics will all draw heavily from this valuable data bank. Demographic data serve as an important framework upon which studies are planned and conducted. Additionally,

these data assist planners in making decisions regarding utilization of resources.

The methods of obtaining census and ongoing surveillance data are unique to Matlab and appear to be adequate. This is emphasized by the fact that collection of these data were continued during the war in 1971 with only an estimated 5% error.

There may be a tendency to over-analyze these data. Better advance planning as to the use of the results of such analyses may be helpful in curbing inappropriate studies. The data concerning the effect of the civil war on population structure do provide some interesting information on the use of mortality rates to portray disasters. The work in neonatal and post-neonatal mortality was well done and provides a very important base for further comparisons of health effects of various intervention activities.

D. Toxoid Field Trial

A field trial of a highly purified glutaraldehyde cholera toxoid, prepared by Wyeth Laboratories was begun in July, 1974. Two doses at 100 mcg each were given 6 weeks apart by jet gun to approximately 43,000 residents of the Matlab study area. Another 43,000 received adult-type tetanus-diphtheria toxoid as control. The cholera toxoid (Lot 20101) was found to be completely free of somatic antigen as judged by the Limulus assay and by inability to evoke vibriocidal antibodies in rabbits. It was administered with an aluminum phosphate-protamine sulfate adjuvant.

At the time of our meeting analysis was complete on the first five to six months of the trial. This encompassed the major epidemic of cholera which peaked in both Dacca and Matlab in mid-October, thus offering an

excellent opportunity for assessing the protective effect of this toxoid.

The results thus far indicate that this lot of toxoid was only moderately and transiently protective. The overall protective level for all age groups was 40-50% during the first nine weeks following the last injection of toxoid and no significant protection was observed during the ensuing 4 months of observation. There appeared to be no significant difference in protection between those who received one and two doses of toxoid, suggesting that the toxoid was exerting a booster effect in most recipients. There was a suggestion of greater protection in children 1-4 years of age, a finding which contrasts with the results of whole-cell vaccine trials in which protection was more marked in adults. This is particularly interesting in view of earlier findings that antitoxic immunity in this population is greater in children than in adults. Since cholera in Bangladesh is primarily a childhood disease, this finding will bear watching to determine whether there is a real age difference in effectiveness of the toxoid, and whether it will be long lasting.

Although larger doses of toxoid or toxoid prepared by a different method or administered by a different route might possibly offer greater protection, the results obtained in the present field trial suggest that a parenterally injected toxoid is unlikely to make a significant long-term reduction in cholera morbidity. The trial, however, did provide a partial and affirmative answer to the question: Does antitoxic immunity play <u>any</u> role in resistance to cholera?

The committee wishes to emphasize that the trial protocol and its execution have been of very high quality and the results of great scientific interest and importance. They have revealed that circulating antitoxin at

levels usually present during convalescence from natural cholera (when volunteers have been shown to be resistant to challenge) may offer little if any protection. This finding alone has taught us a great deal about mechanisms of immunity in enteric disease and especially in toxin-mediated diarrheas.

Therefore, importance of the trial extends far beyond cholera and will provide the essential background for future basic investigation of immune mechanisms in other laboratories. The important point is that this answer could not have been gained in any way other than a properly and rigidly controlled field trial in man in a country where natural cholera occurs with a relatively high frequency. Moreover, a field trial of this high quality could not have been conducted without prior establishment over many years of the Matlab area as a uniquely documented demographic base. Although it has been meticulosly developed and nurtured over the past decade for the chief purpose of evaluating immunoprophylactic agents, it has great potential for other studies useful to Bangladesh extending far beyond the evaluation of vaccines. None of the excellent and valuable demographic data would have been gathered, however, nor would the potential for new population studies exist, were it not for the fact that it was initially developed for the purpose of vaccine evaluation.

The results of this year's toxoid trial point to the need to continue basic studies on cholera immunity, particularly local intestinal immunity. Investigation requiring cholera patients can be uniquely carried out at CRL. These should include studies on the local intestinal antibody response, both antitoxic and anti-somatic, during convalescence from cholera. In

coordination with studies on the induction of local immunity in volunteers in the U.S., it will be important to compare the immune response to oral antigens, both toxoids and somatic, in residents of Bangladesh who have often been previously infected with cholera vibrios. Such studies could be correlated with work on the relation between nutritional status and immune competence. It is now clear that levels of serum antibodies may be poor indicators of protection, especially if these are determined by the vibriocidal assays, since protection appears to be associated chiefly with the IgA class of antibody which has no activity in this test.

Determination of serum antitoxin levels on an approximately 1 in 33 sample of the field trial population has amply demonstrated that 100 mcg of toxoid given once or twice evoked mean circulating antitoxin levels (50-100 AU/ml) similar to those found in individuals convalescent from cholera. Toxoid recipients who developed clinical cholera during this trial were shown to have antitoxin levels in this same range on the day of admission, indicating that they did not represent persons who had failed to respond to toxoid. This again suggested that such protection as was seen was probably not associated with the circulating antitoxin evoked but rather with local antitoxic immunity.

The committee wishes to emphasize that the laboratory support of the field trial, both bacteriologic and serologic, has been excellent. The development of the capability of reliably assessing antitoxin levels on finger-prick blood samples by two methods has provided data essential to the proper assessment of the toxoid. This is the kind of immunologic capability which is appropriate to the needs of CRL and should be highly

commended. Efforts to perfect methods for the measurement of gut lumen antibodies, both antitoxic and anti-somatic, should be encouraged.

III. Plans for the Future

A. Field Trials of Immunizing Agents

The current field trial of toxoid will be carefully followed as originally planned except that the one-year booster will not be given. In view of the modest and short-lived protection afforded by this batch of toxoid during the first few months, it was considered that a one-year booster was unlikely to provide useful information. Meanwhile, energy should be directed toward research efforts which will seek a better understanding of local immune mechanisms before future field trials are launched.

The committee is in complete agreement with this approach,and feels that in the immediate future emphasis should be placed on research in animal and volunteer models, including studies on the local intestinal antibody response (both antitoxic and antibacterial) in diarrhea patients at the CRL.

If and when such studies suggest new and promising immunizing agents or procedures, pilot studies and ultimately field trials can be carried out. The key resource which makes such planning possible is the Matlab study population, which can meanwhile be very effectively utilized for a wide variety of other studies.

It will, however, be important to see whether any protective effect from the toxoid given in 1974 persists until the expected epidemic in the fall of 1975, especially in children. For this reason, the committee wishes to emphasize the need to follow the present field trial population

with the same high quality of surveillance at least through early 1976.

B. Development and Evaluation of Oral Therapy

The Director has proposed that the CRL become engaged in studies designed to improve oral therapy preparations. This would involve development and testing of more acceptable and cheaper preparation methods and packaging techniques with the aim of developing a final product which will be more widely available throughout the world. The CRL is ideally suited to this kind of study because of its unique access to large numbers of patients with acute diarrheal disease. It was understood that emphasis would be placed upon techniques of packaging and evaluation of acceptibility of different colors and flavors which might lead to more effective use. Production and packaging would be carried out in cooperation with a local pharmaceutical manufacturer.

The committee feels that it is proper that the CRL be interested in this kind of developmental activity. There is a genuine need to develop a ready-to-dissolve packet of salts and glucose for making an oral solution which could be widely available throughout Bangladesh. Improvements which are effective here should have worldwide application. If properly distributed and utilized, such a product may not only reduce the number of patients who come to the hospital with dehydration, but may actually reduce mortality from cholera and other acute diarrheas.

It seems likely that the ingredients, although widely available, may not be suitable for direct packaging as some of them may "cake" in this hot and humid environment. Proper selection of inert coloring as well as acceptable flavoring agents will be important in making such packets more acceptible. All these problems seem to be closer to the pharmaceutical industry than to the capabilities of the CRL, therefore competent help from

industry will be essential. The role of the CRL seems better suited to assessment of effectiveness and acceptibility at the bedside and in the field.

For this purpose the Matlab field trial area could be used. Since Matlab now enjoys better medical coverage than much of rural Bangladesh, however, field testing in another previously unstudied area should probably also be included. A major criterion for evaluation should be reduction in mortality as well as reduction in the number and severity of dehydration of patients coming to the center for treatment.

It should be clearly understood that the glucose-electrolyte packet is to be used mostly by the poor, and therefore large profits should not be the motivating factor for industrial involvement.

The success or failure of a readily available oral solution in making an impact on diarrhea mortality will ultimately depend upon education as to its proper use. Perhaps as a first step, solutions should be tested in Rural Health Centers after employees are trained to give it under minimum supervision. Once its effectiveness is proved in these centers, it would be possible to sell it on the open market. Training of health center personnel should probably be centralized. CRL may be able to assist in setting up such training areas.

The committee is concerned that if sufficient emphasis is not given to proper education and supervision, widely available oral solutions may not only fail to achieve the intended objectives, but may actually do more harm than good. They may make parents think that a child with diarrhea is receiving proper care, and they may therefore act with less urgency in taking a severly dehydrated child to a physician or treatment center.

C. Environmental Epidemiology

In connection with ongoing studies of urban cholera and "hot spots" in rural endemic cholera, proposals were offered for employing membrane filtration techniques to more precisely define some aspects of the ecology of cholera vibrios in the environment. Previous studies have been largely qualitative, and these newer techniques offer the opportunity to study vibrio populations on a more quantitative basis. We think such studies would be worthwhile, but may be of limited value unless they are combined with measurements of other environmental factors which function as determinants of vibrio populations. We certainly agree that very little is known in this important area, and that the Matlab study area offers an ideal opportunity to get some answers, because the environmental data can be correlated with epidemiologic data already being collected.

IV. Assessment and Recommendations

A. General Assessment of the Scientific Program

As we have noted before, the staff of the CRL have had to cope with a large number of difficult problems in re-establishing the laboratory during the past year. It should be emphasized that our criticims are meant to be constructive and are made with full recognition of the day to day practical problems which have often forced investigators to relegate scientific activities into a position of lower priority.

We want to emphasize that the Director is first and foremost a scientist, and that he should be afforded greated opportunity to exert scientific leadership and to engage in scientific activity. To this end we urge the recruitment of an expatriate administrative officer who can relieve the

Director of many of the activities which now detract from his primary responsibilities to the CRL.

The committee feels that the presentations during the current meeting and the discussions with individual investigators revealed a certain lack of detailed planning of many of the scientific protocols, and, more particularly, a lack of critical exchange between investigators during this planning. Too many staff members seem to work in isolation. This applies to both CRL staff and JHCMR guest investigators. This lack of exchange of ideas and information is often combined with a lack of familiarity with pertinent scientific literature which has weakened protocol design. There are also some signs of the lack of a spirit of institutional excellence, and too much of a feeling of pursuit of private goals.

The committee strongly recommends that a program of regular and more frequent seminars be developed under the Director's stumulus and supervision, and that a system of critical protocol review by an in-house committee be instituted. External review may be desirable in certain instances. The recruitment of an administrative officer should make it possible for the Director to devote more of his time to these critical duties.

This recommendation is not to be taken as a reflection on the present administrative section who are doing a fine job in local affairs. It merely recognizes the need for an individual who can take care of necessary contacts with the United States and local American agencies and thereby give the Director more time for scientific activity.

B. Personnel

The most critical lack at the present moment is a senior clinical investigator who can provide the nucleus of leadership for clinical research as well as provide supervision and guidance to the clinical staff of the CRL hospital. It is essential that the CRL continue to serve as a treatment center of actue diarrheal disease in Dacca. The CRL hospital must always be prepared for unexpected outbreaks of cholera such as the epidemic during the fall of 1974. Even with adequate preparation, such emergencies will always tax the facilities and staff and force temporary changes in priorities. The CRL hospital should be a standard of excellence in patient care in which local physicians can receive the best training.

At the moment the only expatriate clinicians in the CRL are three guest investigators belonging to the Johns Hopkins CMR unit. Two of these will leave in mid-1975. Because CMR staff are guests and do not provide long term continuity, the committee recommends that highest priority be given to the recruitment of a senior clinical investigator. A major role of such an investigator will be the training and development of talented local physicians who can eventually take charge of clinical investigation at the CRL.

The need for an administrative officer is discussed above.

Attempts should be made to provide more training of medical and paramedical personnel, with the ultimate aim that the laboratory will eventually be staffed entirely by Bangalee personnel. Efforts should be made to increase the amount of advanced training in foreign countries. WHO fellowships should be sought.

C. Scientific Program

A number of recommendations and opinions concerning specific scientific projects are expressed in the earlier portion of this report. Listed below are selected areas to which we think high priority should be given, and brief summaries of our assessment of certain areas of investigation:

1) Nutrition, Immunity, and Infection

The committee strongly supports plans for increased scientific activity in the area of the impact of nutrition on immunologic competence and infectious disease morbidity. We applaud the beginnings in this direction which were made during the past year. The Matlab field trial area lends itself well to such studies and should be fully exploited to these ends within the capacities of the scientific staff to maintain standards of high quality. The unique demographic base offered by Matlab is a first-rate resource almost without equal in the world. It would require at least 10 years to achieve a similar body of fact and understanding about a people as has been achieved by the CRL staff during the past decade in this part of rural Bangladesh. The maintenance of the Matlab study area should be the Number One priority of the CRL. The continued maintenance of Matlab is essential both for extension of scientific activity into the relationship between nutrition, immunity and disease, but also for the future study of new vaccines as they become available for field trial. These can be tested only if this study area is maintained. We see this broadening of the use of the Matlab population as a very positive step which will increase its potential for making scientific contributions of public health value.

A strong back-up facility in the CRL hospital and laboratories in Dacca will remain necessary, however, if the quality of work in Matlab is to be maintained. Certain kinds of work can be done only in Dacca. An example of

this would be studies on local immune mechanisms. Such studies should be related to and stimulated by field observations made in Matlab, but the facilities required for the two kinds of investigation differ greatly.

While the scientific program is being expanded to include more nutritional studies, we feel that it will be necessary to keep the focus on diarrheal disease as a major theme. There is still much to be learned about cholera and diarrhea. The expertise of the CRL staff and its reservoir of experience lies in work in this area. Diarrheal disease should be the major infectious disease group to be studied in relation to immune competence and nutritional status.

We would also urge that extension to other areas outside of infection, immunity and nutrition (such as family planning) should be entered with caution. Care should be taken to avoid over-use of the Matlab population. Studies must be carefully planned and supervised and conducted in such a way as not to disturb either the population or the ongoing basic projects. To allow inexperienced and unsupervised workers to come into the area and do random ad hoc projects in unlikely to be rewarding and likely to be counter-productive. Projects should not be undertaken without detailed, written protocols, including background and rationale, which have received internal and, if appropriate, external peer review. In this connection,we feel that the Director must have authority of approval of all work done under the sponsorship of CRL both in Dacca and at Matlab.

The following represent some of the specific studies relative to nutrition which would be appropriate as CRL activities:

Studies which would be of value, both to Bangladesh and to other countries, would be further documentation of the relationships between nutrition and disease, including functional and behavioral development, accurate evaluation of calorie and protein intakes relative to growth and development, and infectious

disease morbidity. There is need to establish by chemical procedures whether the prevailing nutritional deficiencies are of protein and calories or whether Vitamin A, iron and possible folic acid are involved.

The most immediately useful studies which could be done at the CRL in Dacca would be determination of the extent to which enteric infections cause chronic impairment of absorption of protein and other essential nutrients. It is likely that food requirements in Bangladesh are increased by diarrhea, but no data are available from Bangladesh, and very little from other countries. This would require the feeding of a standardized diet, and the measurement of dietary and fecal nitrogen over a period of several days. Absorption tests and measurement of fecal fat could be included. The study of nutrient losses during acute infection would be technically more difficult, but useful.

2) We recommend that when appropriate investigators become available, studies begin on immune mechanisms in cholera and related acute diarrheas, with special emphasis on local intestinal immunity. These were begun in the 1960's and interrupted due to discontinuation of the research program. Since that time a great deal has been learned about local antibacterial and antitoxic immunity in animal models. The CRL offers an ideal opportunity to resume these studies, with special emphasis on function rather than immunochemical characteristics. The relative ineffectiveness of a parenterally injected toxoid in the current field trial emphasizes once again the need for such studies. They should involve comparisons of systemic and local antibody response in disease and following toxoid administration both by parenteral and oral routes. Technical competence already exists in the CRL staff for the measurement of antitoxic and vibriocidal antibodies.

3) Oral Sucrose Studies -

A well-controlled study comparing glucose and sucrose in oral therapy should be carried out as soon as possible.

4) Bacteriology -

Consideration should be given to greatly reducing routine stool cultures on non-study cases in the hospital, and spending more time developing typing methods, especially in preparation for future environmental studies in which the recognition of a larger number of markers will be required.

5) Shigellosis studies -

Studies on the exotoxin(s) produced by the local strains were not presented at the meeting. This work should be vigorously pursued, and comparisons made with Guatemalan strains of Shiga bacillus associated with recent epidemics. The frequency of bacteremia badly needs documentation, both in leukemoid reactions and in typical cases. The study of changes in T and B cells in the leukemoid response should be continued.

The protocol for the proposed shigellosis project in Teknaf seems vague and over-ambitious. Shigellosis, a nationwide problem in Bangladesh, is of great public health importance. It certainly merits investigation. However, it is doubtful whether a study so far from Dacca represents the most efficient way for CRL investigators to study the disease. The Teknaf project will need critical evaluation next year.

D. Recommendations for Future Meetings of the Technical Committee

The committee recognized that the CRL and its staff have faced special difficulties this year because of the short notice it was given for preparation for the meeting, shortage of experienced staff,and the massive effort devoted to the large cholera epidemic of the past fall. However, we feel

we should suggest steps which should be taken to provide for a smoother, more informative and more effective meeting next year.

1. The following materials should be mailed to the committee members before they leave home for Bangladesh:
   a) The table of organization and list of staff members and titles.
   b) Abstracts of all papers to be presented. Full manuscripts would be useful if available this early.
   c) Brief summary of previous year's major events and major research areas and goals.
   d) Summary of research in progress which will not be presented during the program.
2. Full manuscripts should be provided at the meeting, especially if detailed tables and graphs are included in the presentation.
3. Presentations of scientific reports by staff members should not begin on the day the committee members arrive.
4. The program should be ordered as follows:
   a) Private introductory session with the Director in which he provides the committee with an outline of research policy and summary of the current research program.
   b) Private meeting of the Technical Committee.
   c) Tour through CRL in Dacca with the Director.
   d) Presentation of individual research reports by staff members with ample time for questioning.
   e) Trip to Matlab Bazar, with presentation of pertinent papers at Matlab hospital. This trip should probably follow all presentations at the CRL in Dacca.

f) Joint meeting of Technical Committee and Directing Council.

g) Two or three full working days following meeting should be allowed for preparation of the report.

h) The program should be arranged so that committee members may have some hours each day to talk with individual investigators.

i) Time should be available so that the committee can meet in executive session for one to two hours each day to discuss presentations and assign responsibility for report preparation.

In general, we feel that less emphasis should be placed on individual presentations of papers and more on private discussions between investigators and members of the Technical Committee. We realize that presentation of data before a group can be an important event to a younger investigator, but we wonder whether it might not be possible to organize group presentations in which all members of the group are on tap for questions, but the "public presentation" is given by a senior person.

Signed by the members of the Technical Committee:

John P. Craig, Chairman
United States Representative

M.A. Latif
Bangladesh Representative

Derrick Rowley
Australian Representative

R.E.O. Williams
United Kingdom Representative